

What efficacy and safety profiles can we expect

*Mathematical and Computational Biology in Drug Discovery
(MCBDD) Module IV*

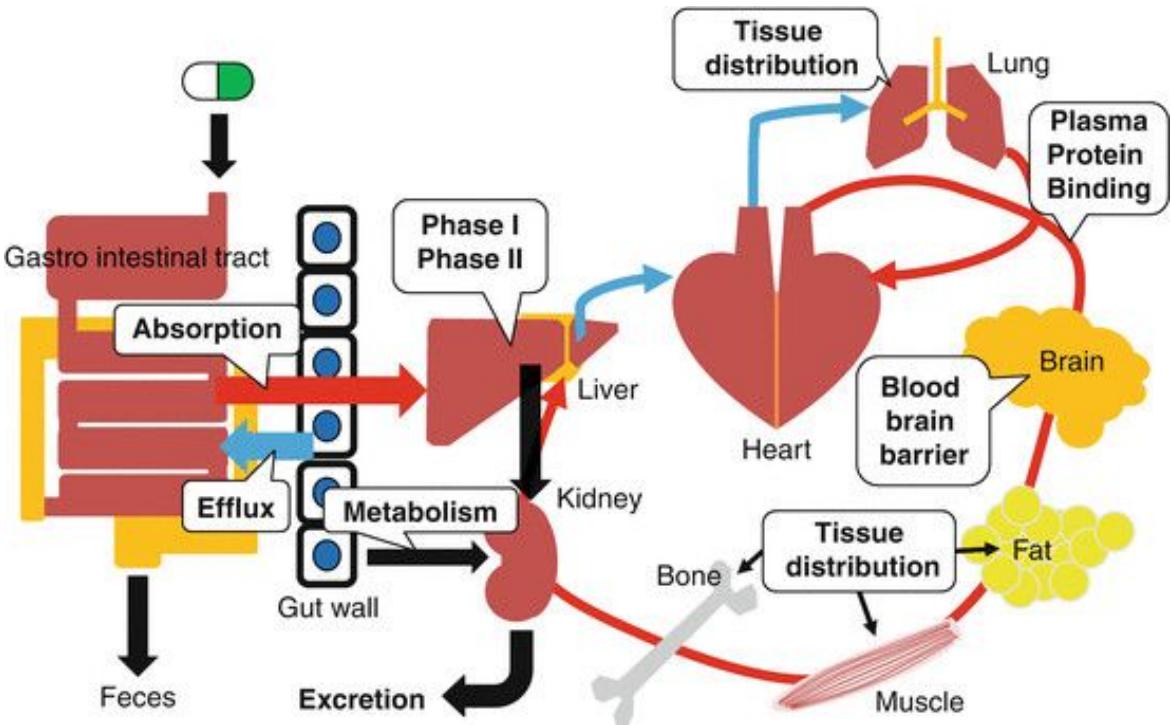
*Dr. Jitao David Zhang
April-May 2021*

The outline of the module

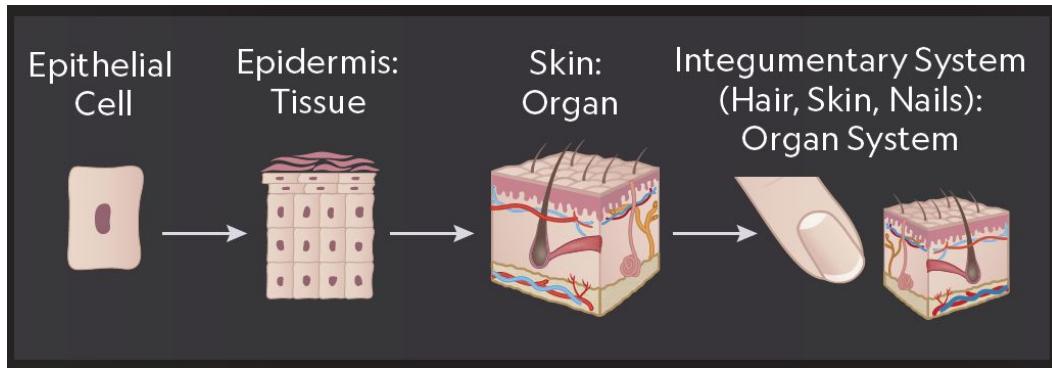
- MoA understanding of the compound with gene expression
 - Single-cell gene expression
 - RNA velocity
 - Spatial transcriptomics
- Target identification
 - CESTA
 - Degradation
 - Binding mode
- Techniques and platforms for MoA understanding
 - Digital pathology
 - Organoids

Factors that affect efficacy and safety profiles

- Absorption
- Distribution
- **Pharmacology**
- **Toxicology**
- Metabolism
- Excretion



Complexity Increases Through a System

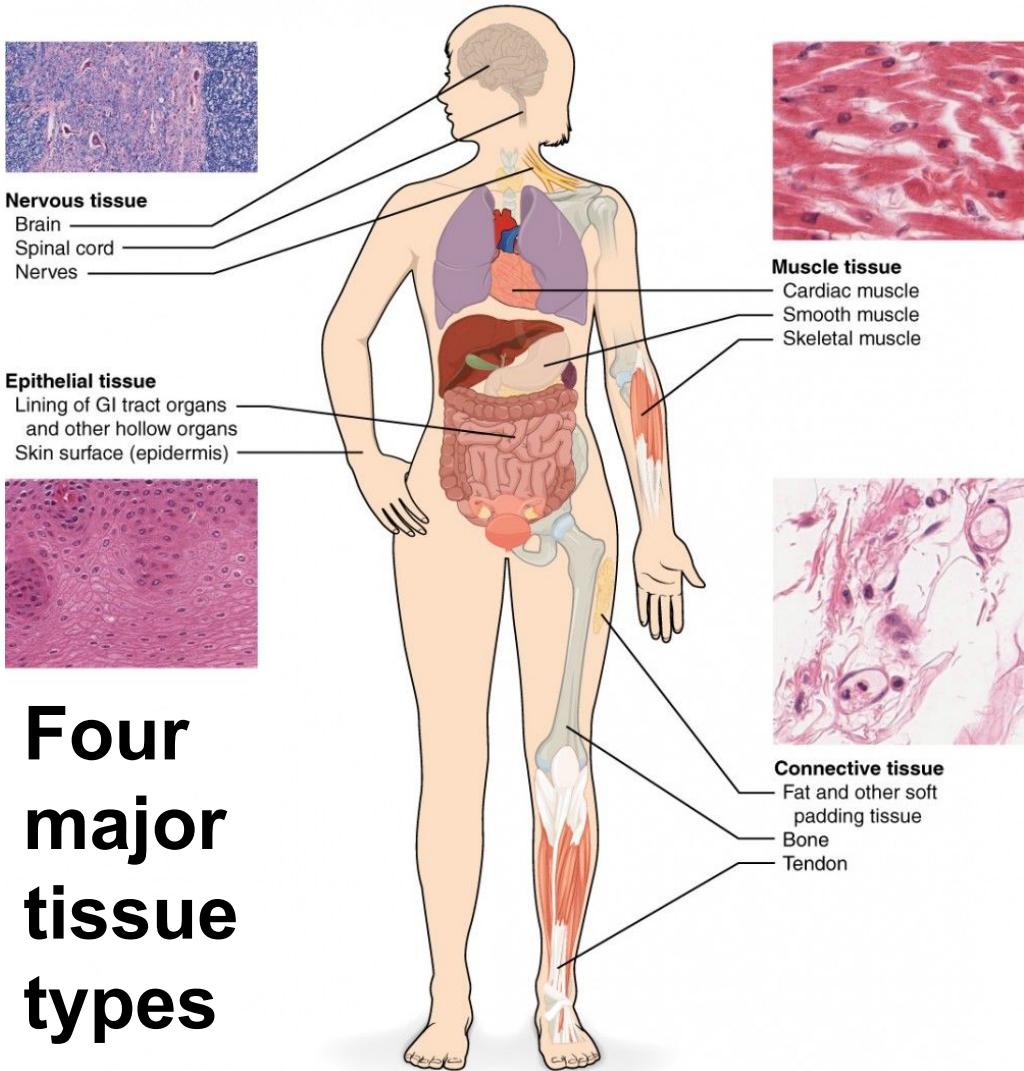
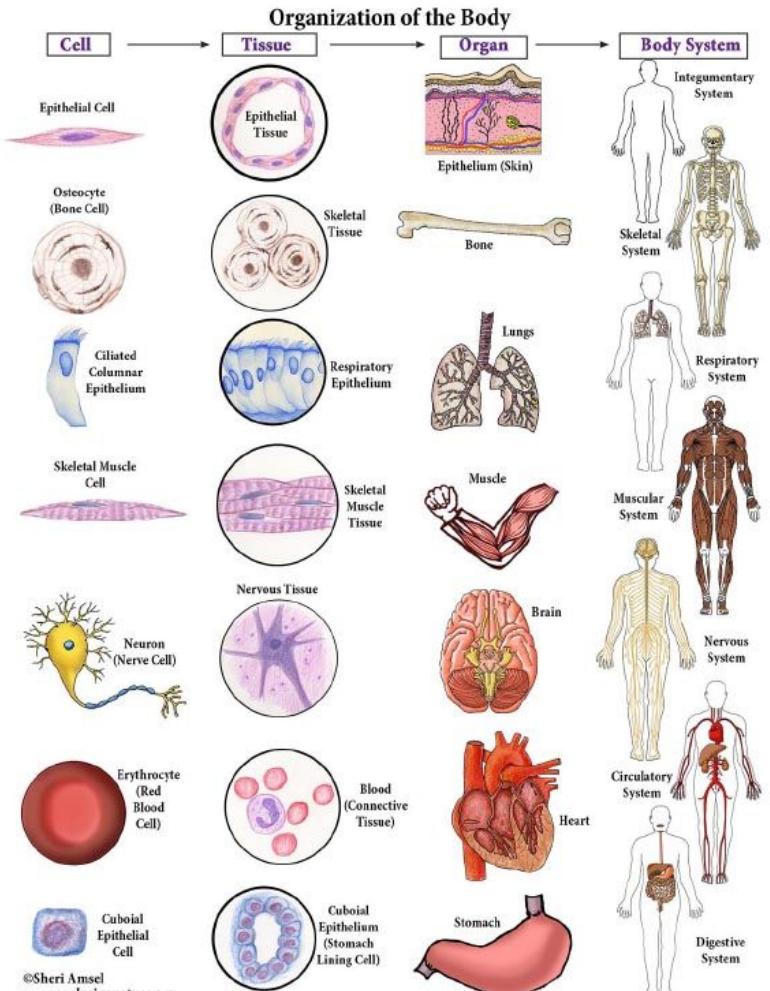


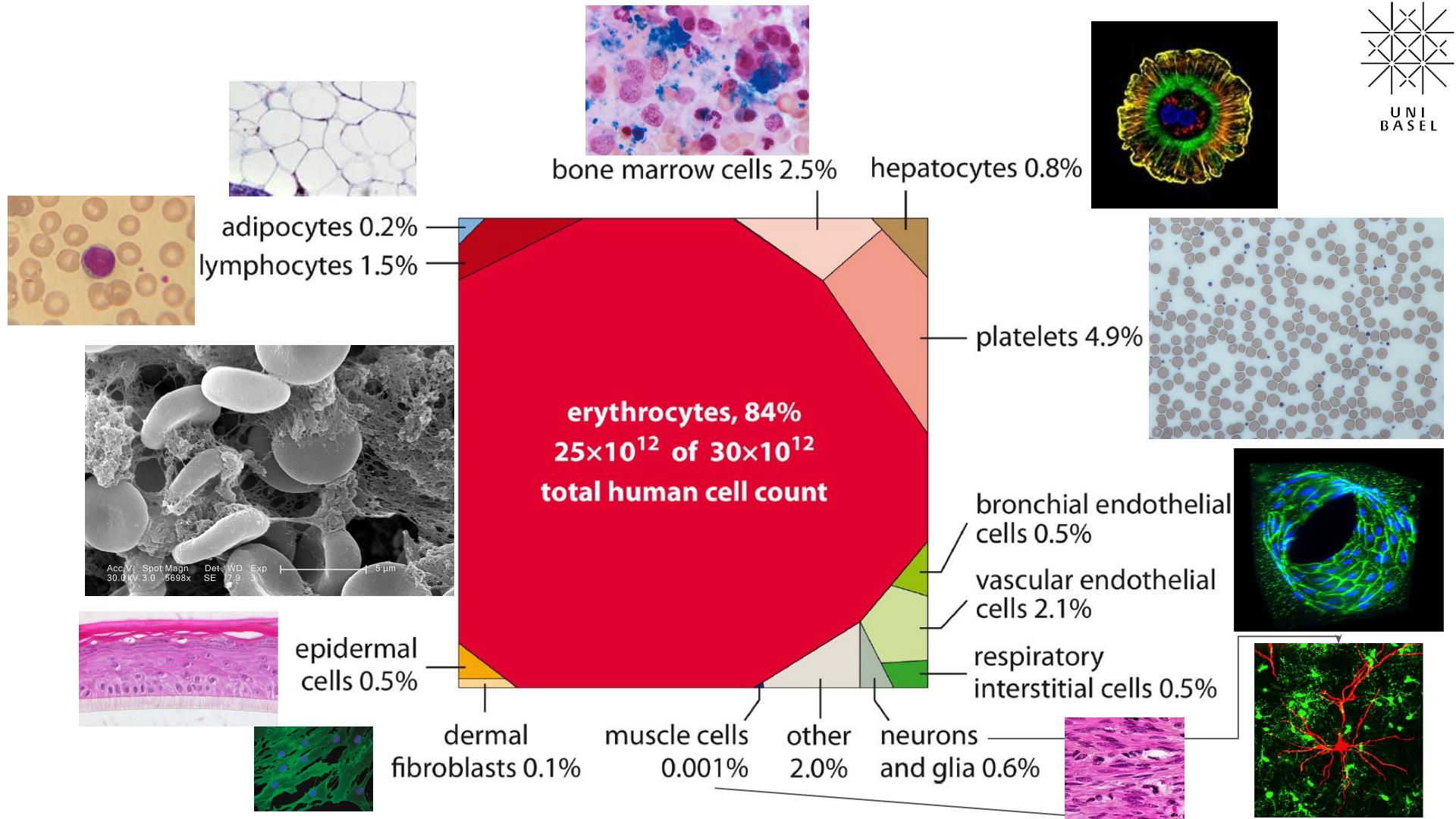
Cells: basic building blocks, of variable morphologies and functions

Tissues: groups of specialized cells that communicate and collaborate

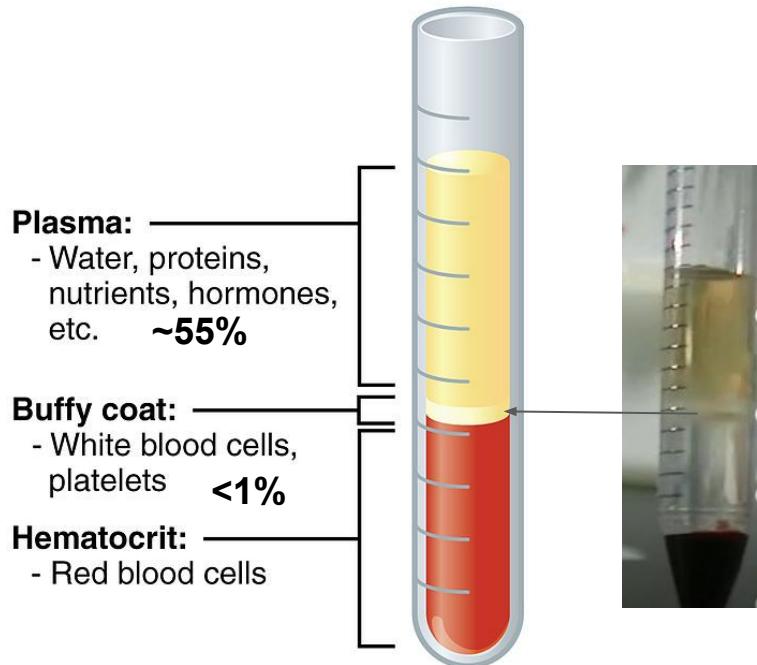
Organ: group of tissues to perform specific functions

Organ systems: group of organs and tissues



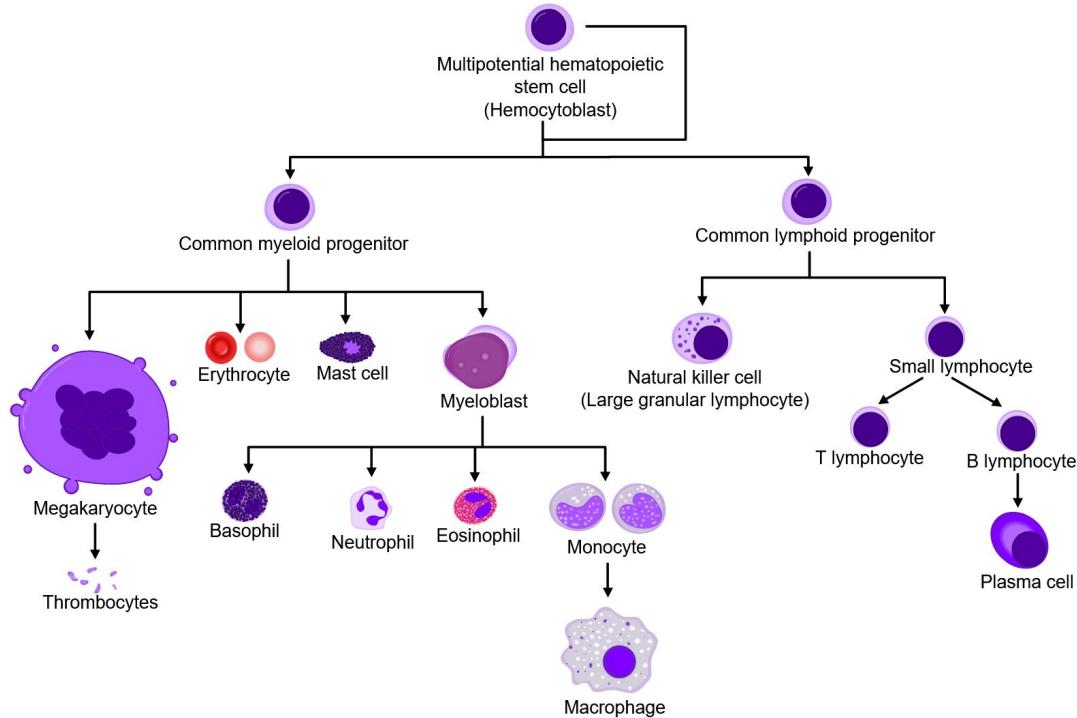


What's in a drop of blood? Ask a doctor!



Normal Blood:

♀ 37%–47% hematocrit
 ♂ 42%–52% hematocrit

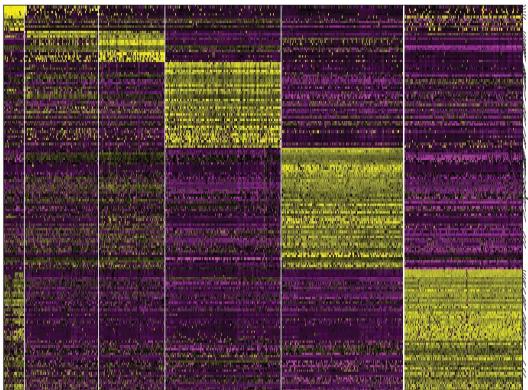


What's in a drop of blood? Count the genes!



Sequencing

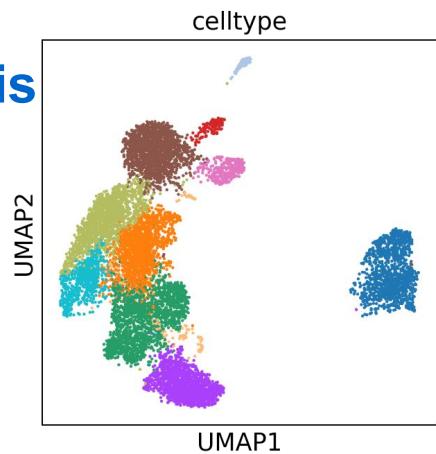
Genes



Cells

Low Expression  High Expression

Data analysis

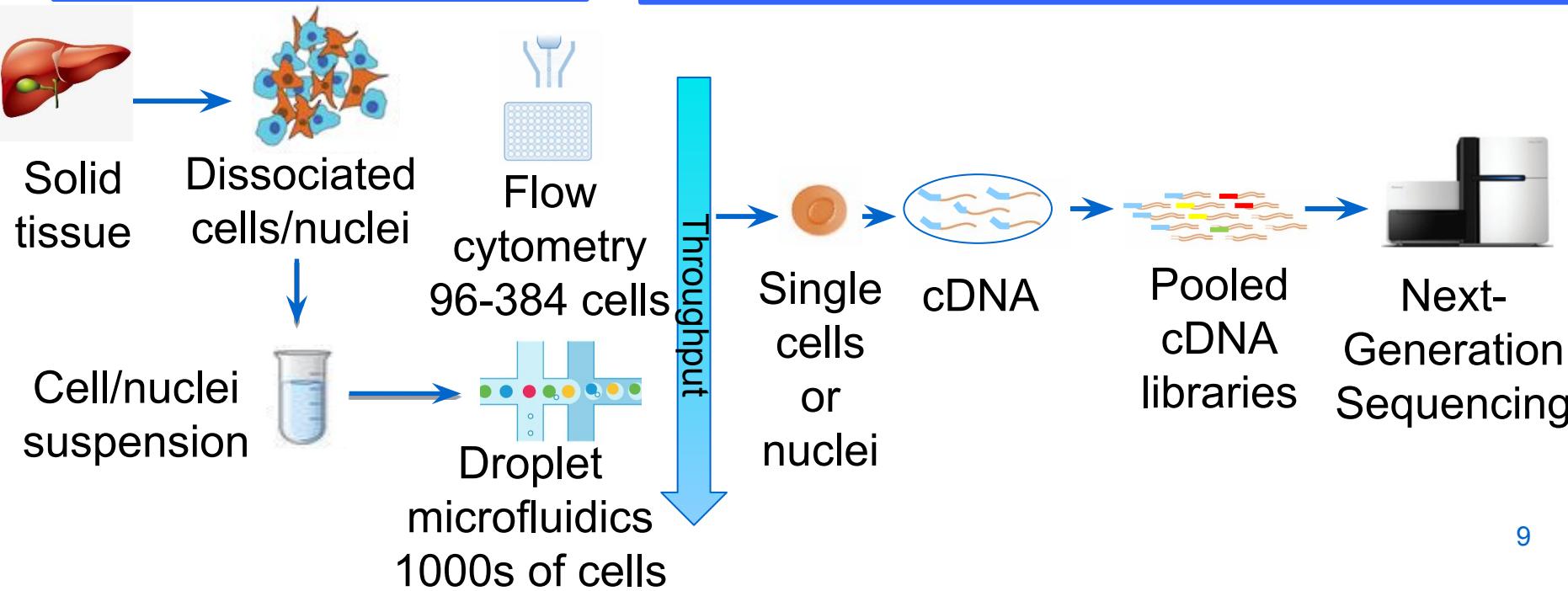


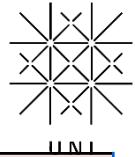
- B-cell
- CD4 T-cell
- CD8 T-cell
- DC
- NK cell
- monocyte CD14+
- monocyte CD16+
- naive CD4 T-cell
- naive CD8 T-cell
- pDC
- unknown

Single-cell sequencing (scSeq) workflow

Tissue dissociation

Single cell capture and transcriptome sequencing



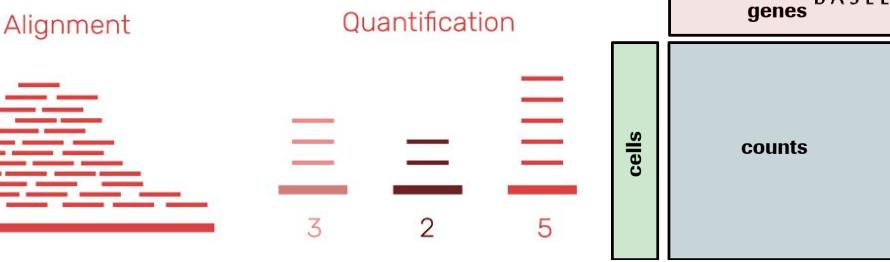


A linearized workflow of scSeq data analysis

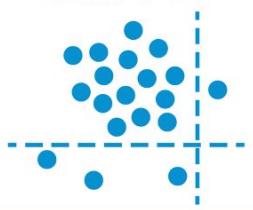
From short reads to gene-cell matrix

QC, filtering & normalization,
dimensionality reduction, and
clustering

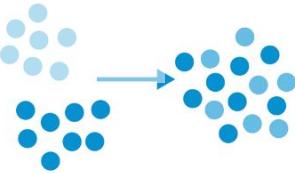
Downstream analysis



Quality control



Normalisation



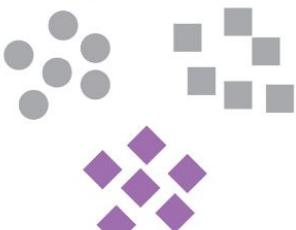
Clustering



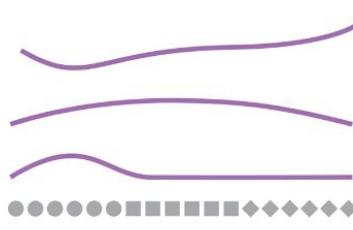
Differential expression



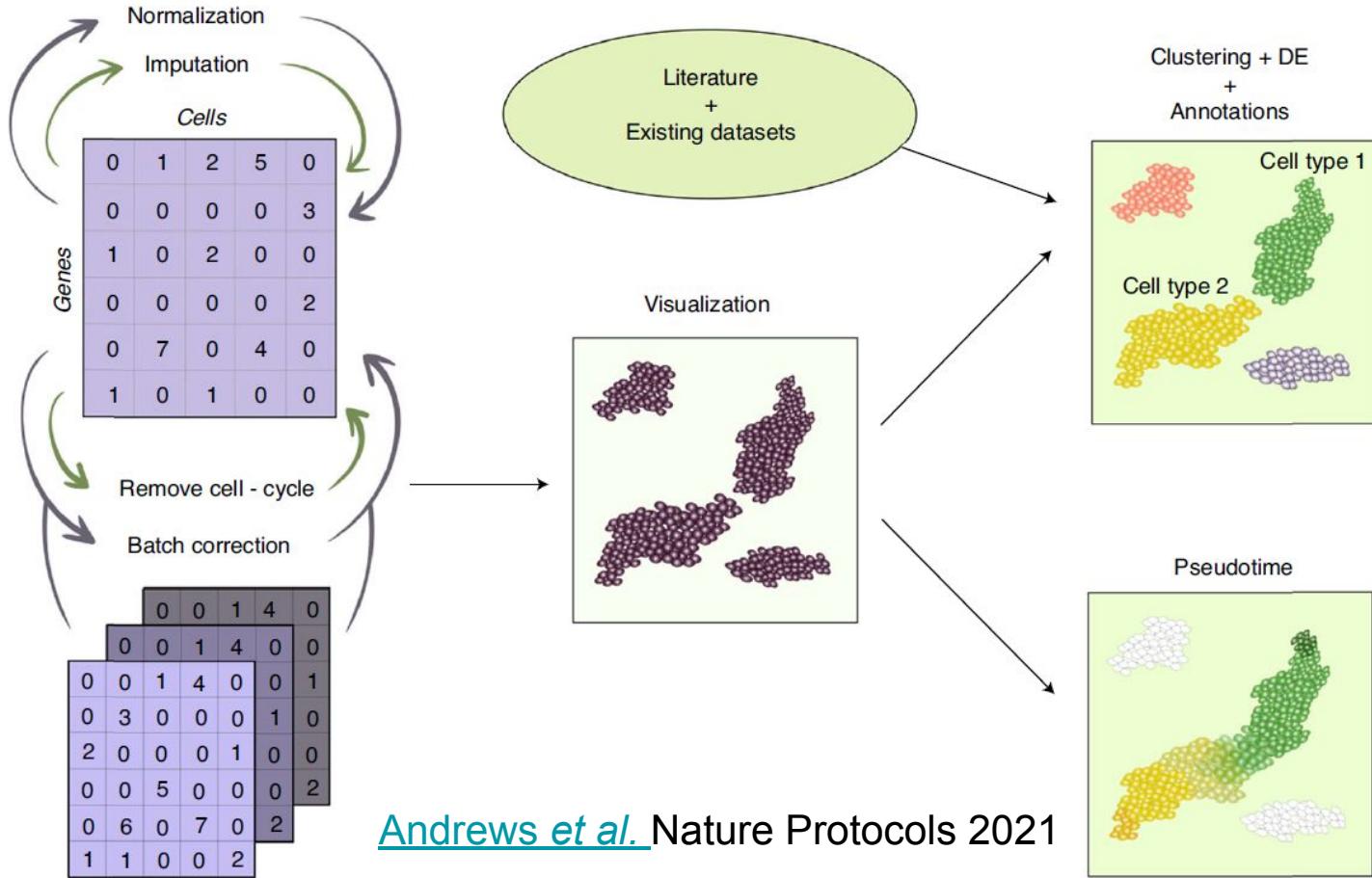
Marker genes



Expression patterns

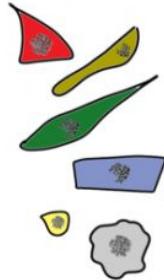
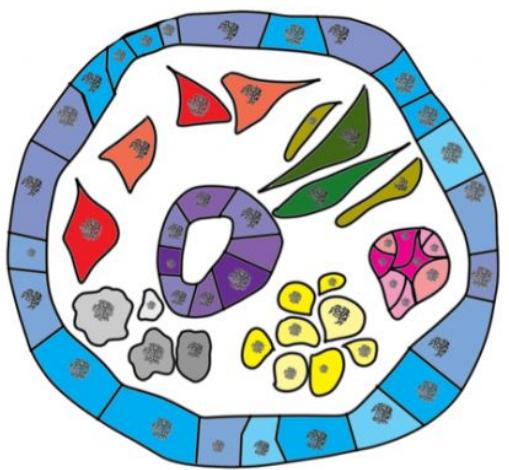


Overview of the computational workflow

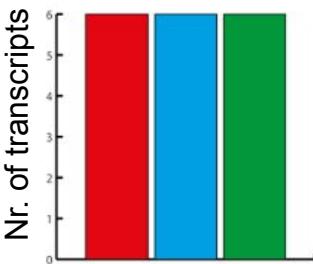


[Andrews et al.](#) Nature Protocols 2021

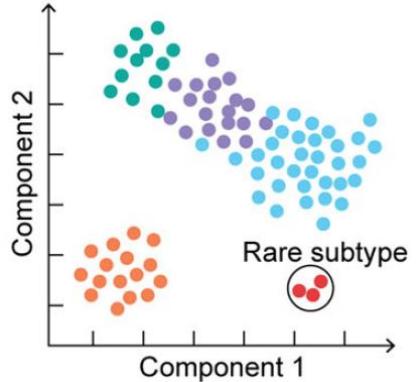
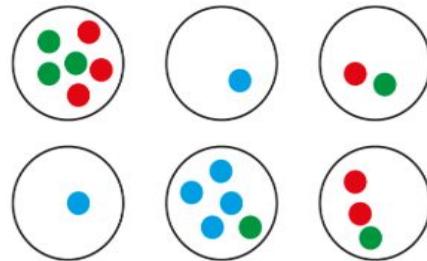
Single-cell biology benefits both disease understanding and drug discovery



Bulk analysis



Single cell transcriptome analysis



Single-cell biology is important in drug discovery

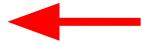
Disease understanding:

disease-specific cell types
and states



Target identification:

expression pattern in
health and disease across
cell types



Biomarker and patient stratification: which genes should we measure in which cell type(s)?



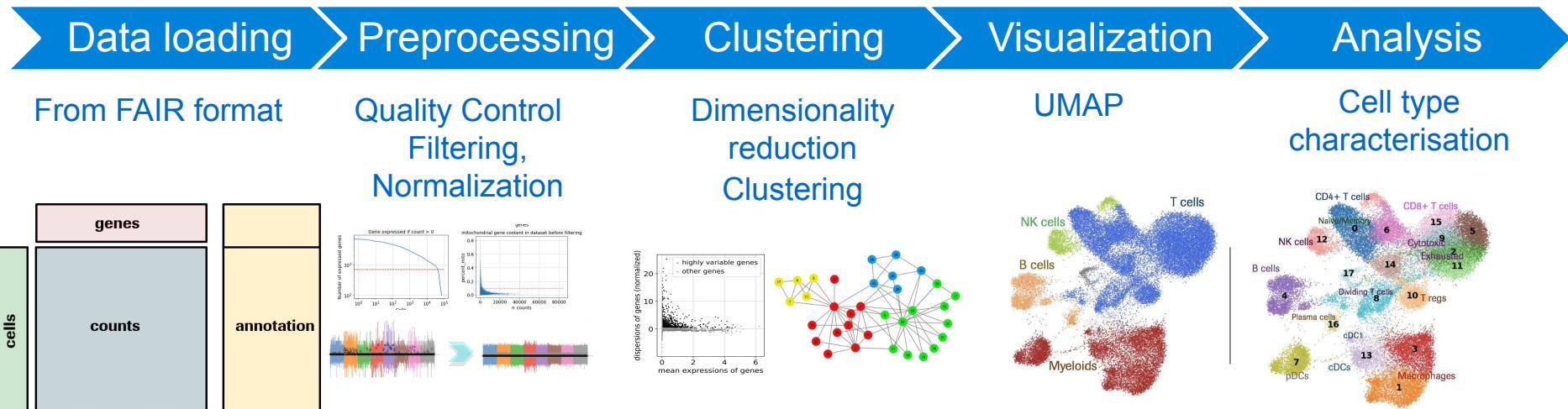
MoA and safety

modelling: perturbation effect at single-cell level



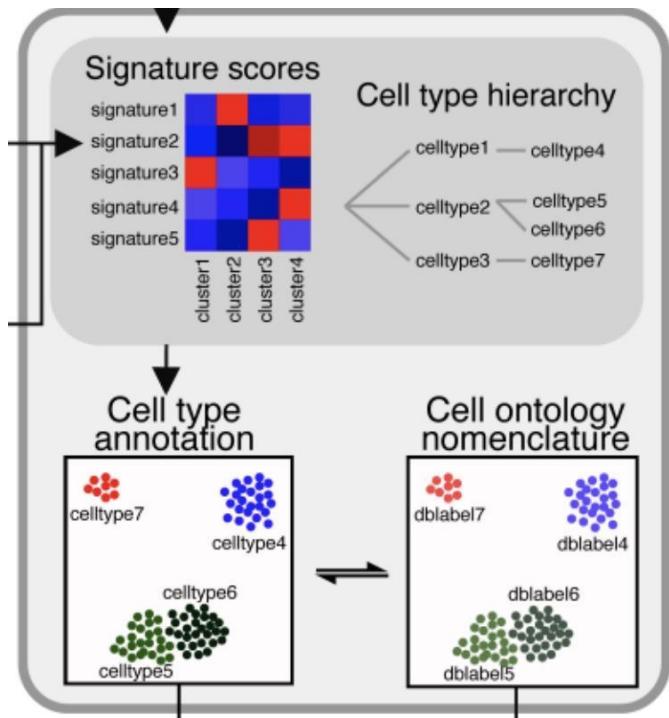
BESCA: An open-source Python package for single-cell gene expression analysis

An automated standard workflow

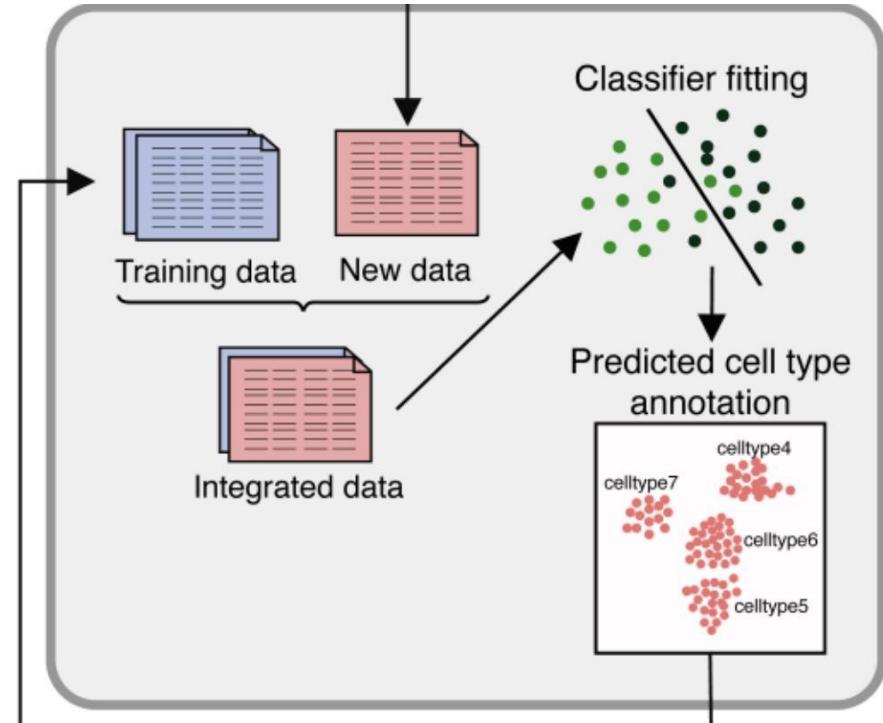


Cell type annotation with machine learning

**Left: Classical,
signature-based annotation**



**Right: annotation with
supervised machine learning**

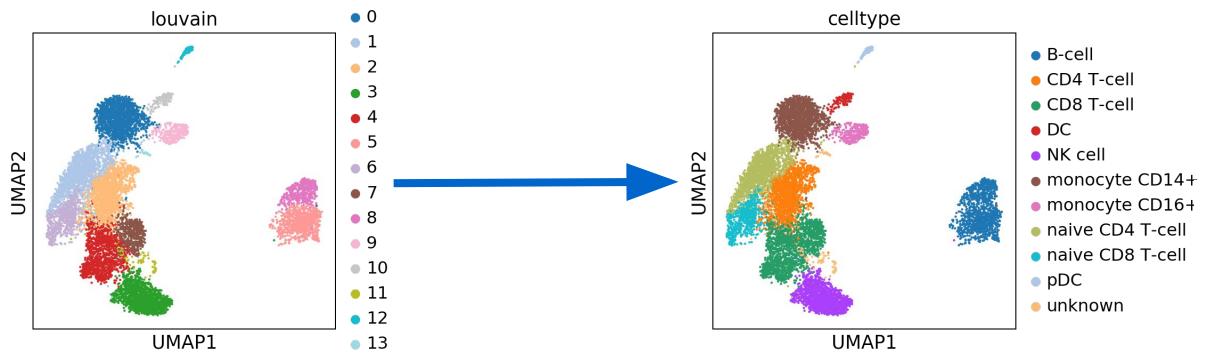


An intern project: Cell type annotation

From unsupervised clustering and cluster based annotation:



Luis Wyss
RAAN intern 2019



	Gene 1	Gene 2	Gene 3	Gene 4	Gene 5	Label
Training Cell 1	10	50	0	12	4	Celltype A
Training Cell 2	8	45	78	3	23	Celltype B
Training Cell 3	14	55	78	65	55	Celltype B
Training Cell 4	78	12	13	9	58	Celltype A
Training Cell 5	45	23	65	98	11	Celltype C

To supervised annotation at single-cell level:

	Gene 1	Gene 2	Gene 3	Gene 4	Gene 5
Cell 1	45	45	8	56	3
Cell 2	65	120	78	45	12
Cell 3	79	12	34	65	88
Cell 4	7	59	32	47	62

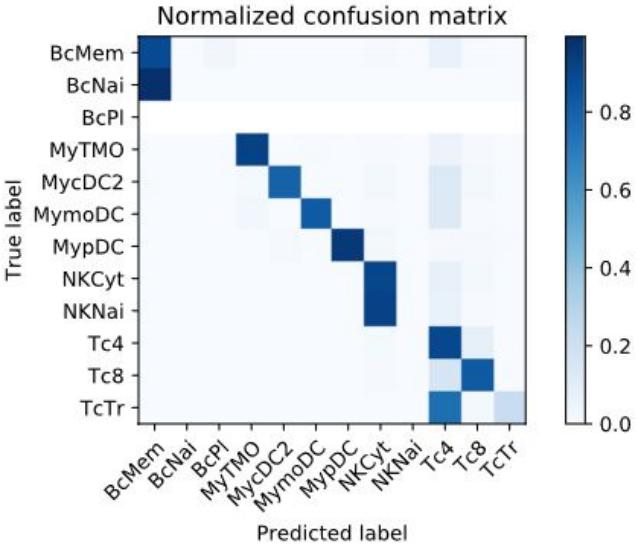
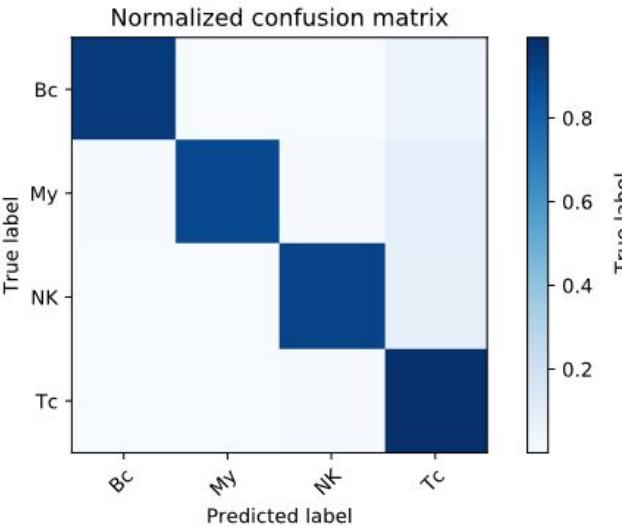
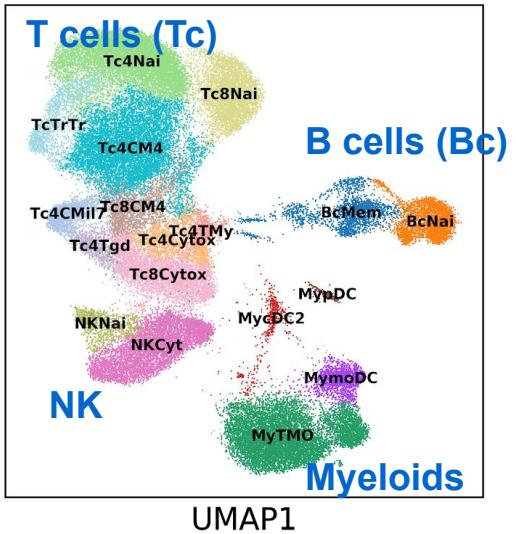


	Gene 1	Gene 2	Gene 3	Gene 4	Gene 5	Prediction
Cell 1	45	45	8	56	3	Celltype A
Cell 2	65	120	78	45	12	Celltype B
Cell 3	79	12	34	65	88	Celltype C
Cell 4	7	59	32	47	62	Celltype B



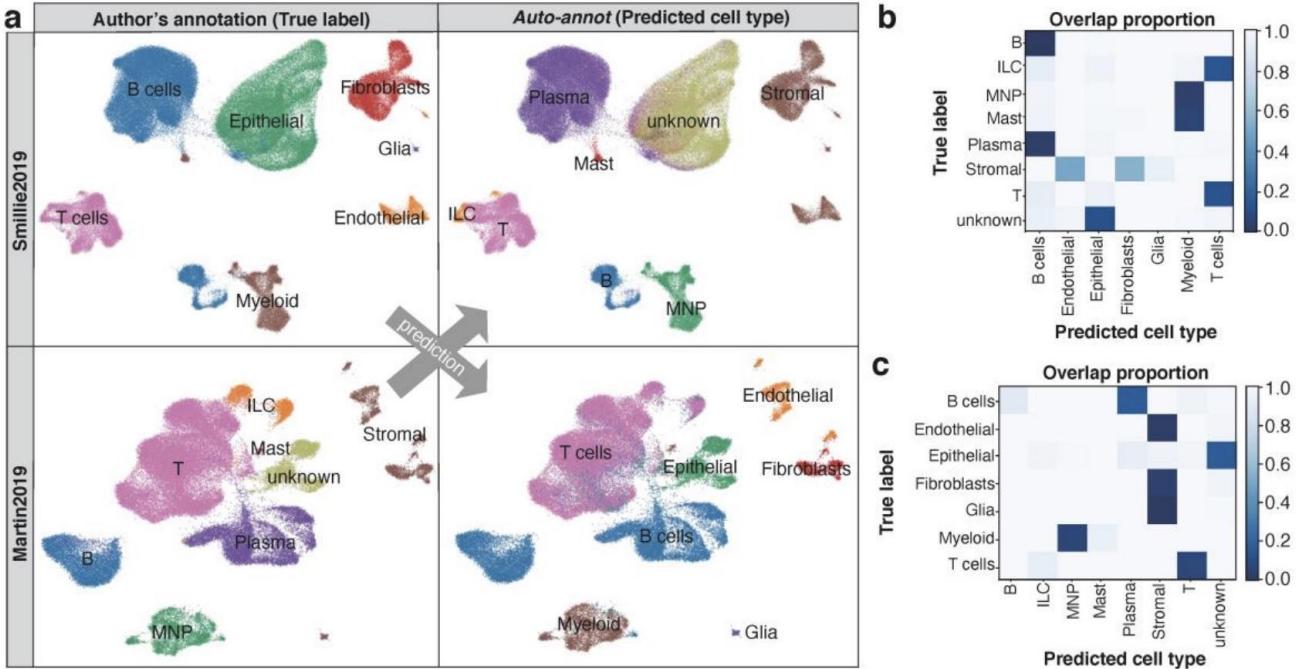
Advantages: (1) automation, (2) annotation independent from clustering, and (3) we can estimate the confidence of prediction

A PBMC example of cell type annotation



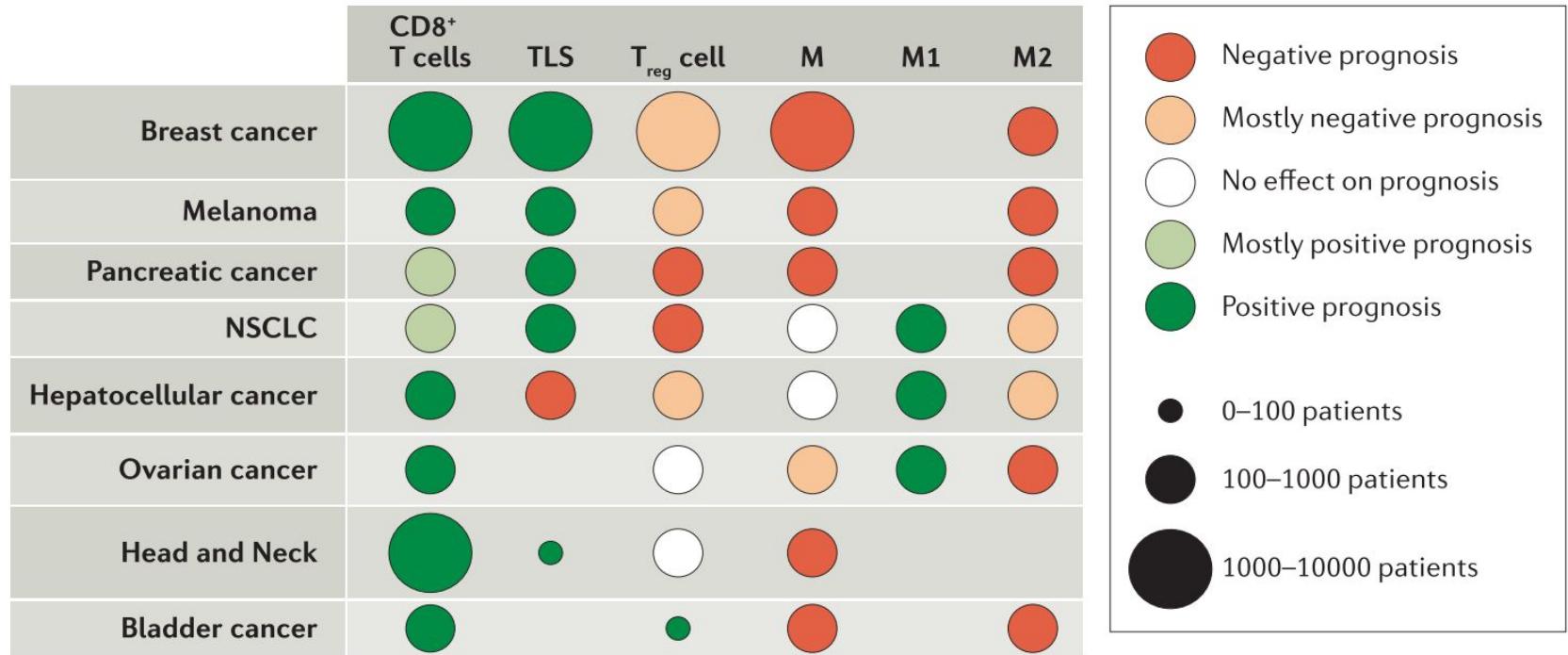
- Broad level cell types, including B cells (Bc), Myeloid (My), NK cells (NK) and T cells (Tc), are successfully predicted.
- Missing and highly similar cell types cause challenges with increased granularity. Essential: reference data quality and knowledge of cell types. 17

An example of Inflammatory Bowel Disease (IBD)



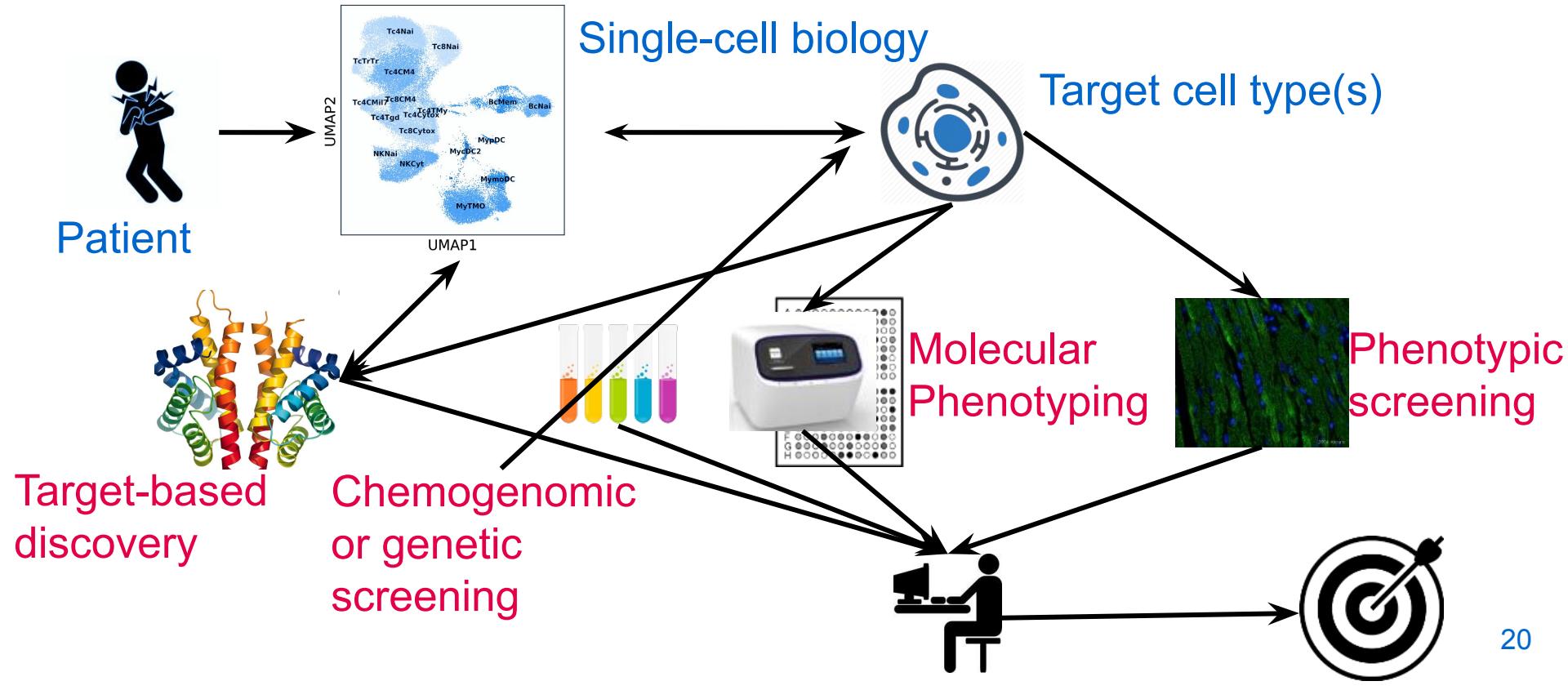
We observed Inconsistent cell type nomenclature across studies.
 Machine learning allows us compare and integrate multiple studies.

Abundance of immune cells in tumor microenvironments affect outcome



TLS: tertiary lymphoid structures; T_{reg}: regulatory T cells; M: macrophages; M1/M2: subtypes of macrophages

Computational biologists work with experimentalists to empower drug discovery



We are living ecosystems

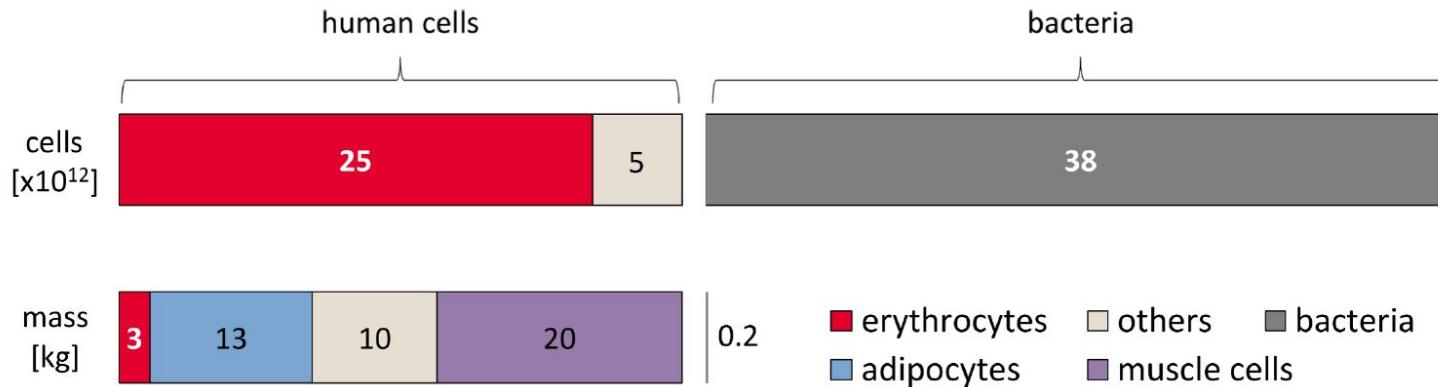
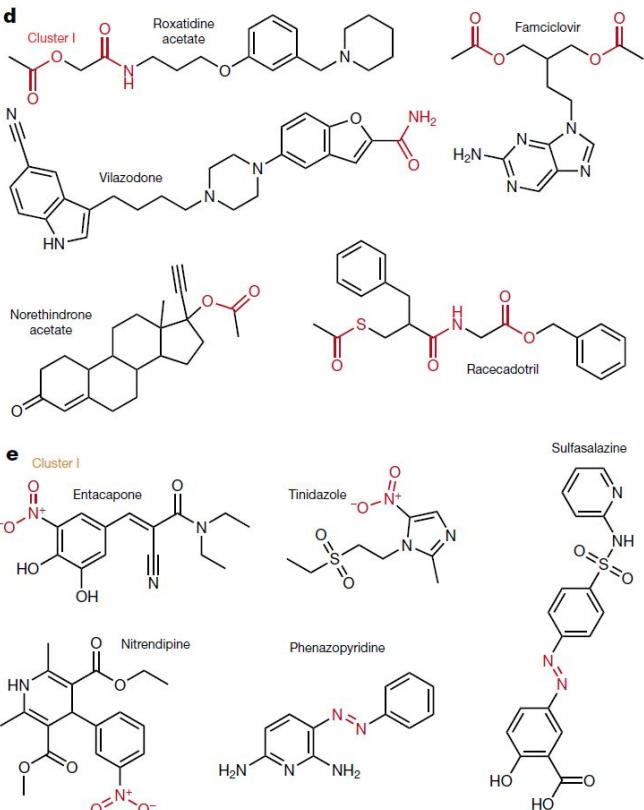
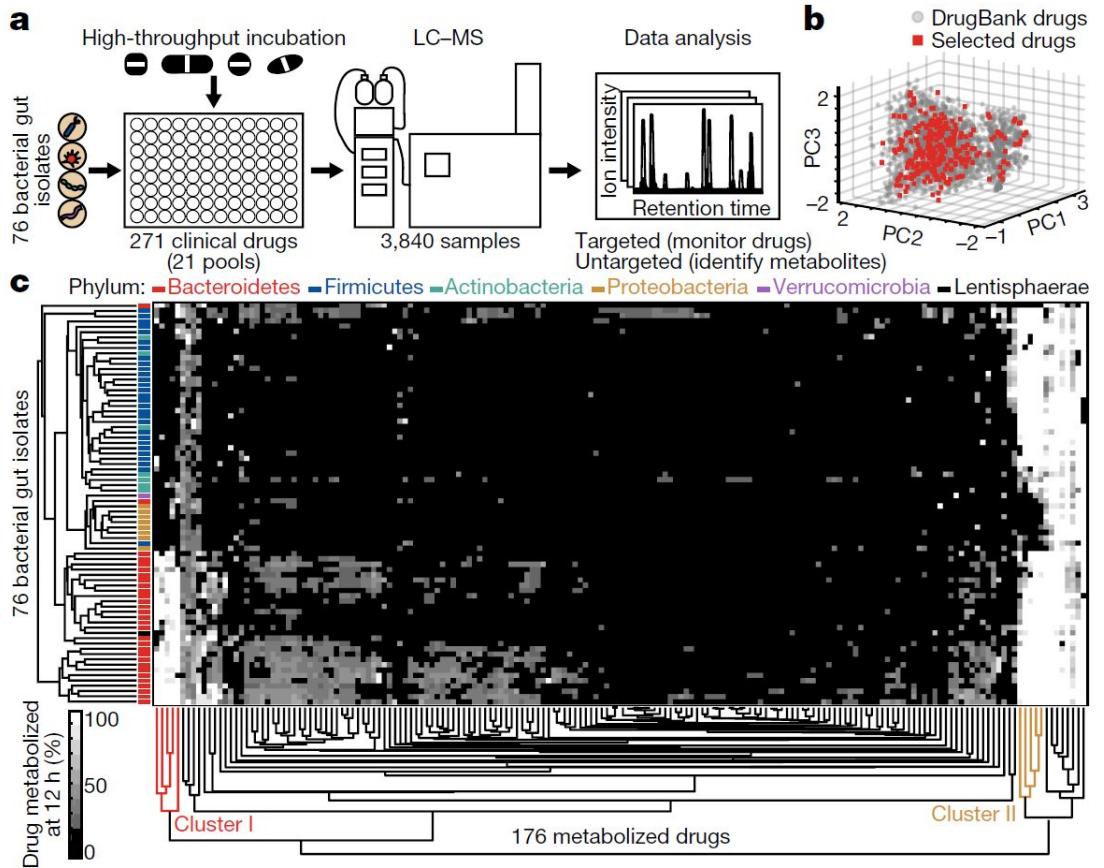


Table 3. B/H ratio for different population. See Table B in [S1 Appendix](#) for full references.

population segment	body weight [kg]	age [y]	blood volume [L]	RBC count $[10^{12}/L]$	colon content [g]	bac. conc. $[10^{11}/g \text{ wet}]^{(1)}$	total human cells $[10^{12}]^{(2)}$	total bacteria $[10^{12}]$	B:H
ref. man	70	20–30	4.9	5.0	420	0.92	30	38	1.3
ref. woman	63		3.9	4.5	480	0.92	21	44	2.2
young infant	4.4	4 weeks	0.4	3.8	48	0.92	1.9	4.4	2.3
infant	9.6	1	0.8	4.5	80	0.92	4	7	1.7
elder	70	66	3.8 ⁽³⁾	4.8	420	0.92	22	38	1.8
obese	140		6.7	5.0 ⁽⁴⁾	610 ⁽⁵⁾	0.92	40	56	1.4

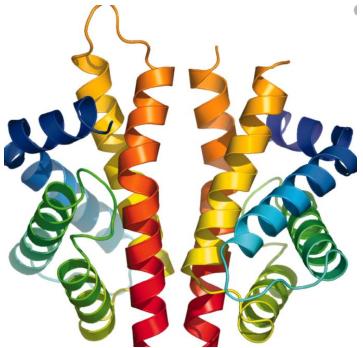
Gut microbiome can metabolize drugs differently



Conclusions

- Single-cell biology allows us to identify rare cell populations associated with diseases, and investigate cell-type-specific perturbations caused by drug candidates.
- Algorithms for dimensionality reduction, clustering, and semi-automated cell type annotation allow us interpret and integrate single-cell datasets.

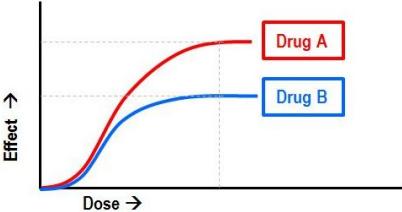
To understand protein function is critical for drug discovery



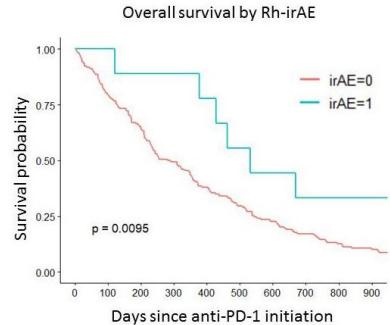
Protein function



Cellular phenotype



Drug efficacy and safety

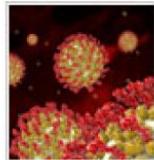


Clinical outcome

ONCOLOGY



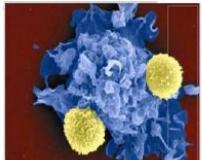
INFECTIOUS DISEASE



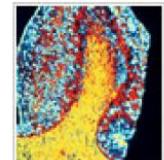
IMMUNOLOGY



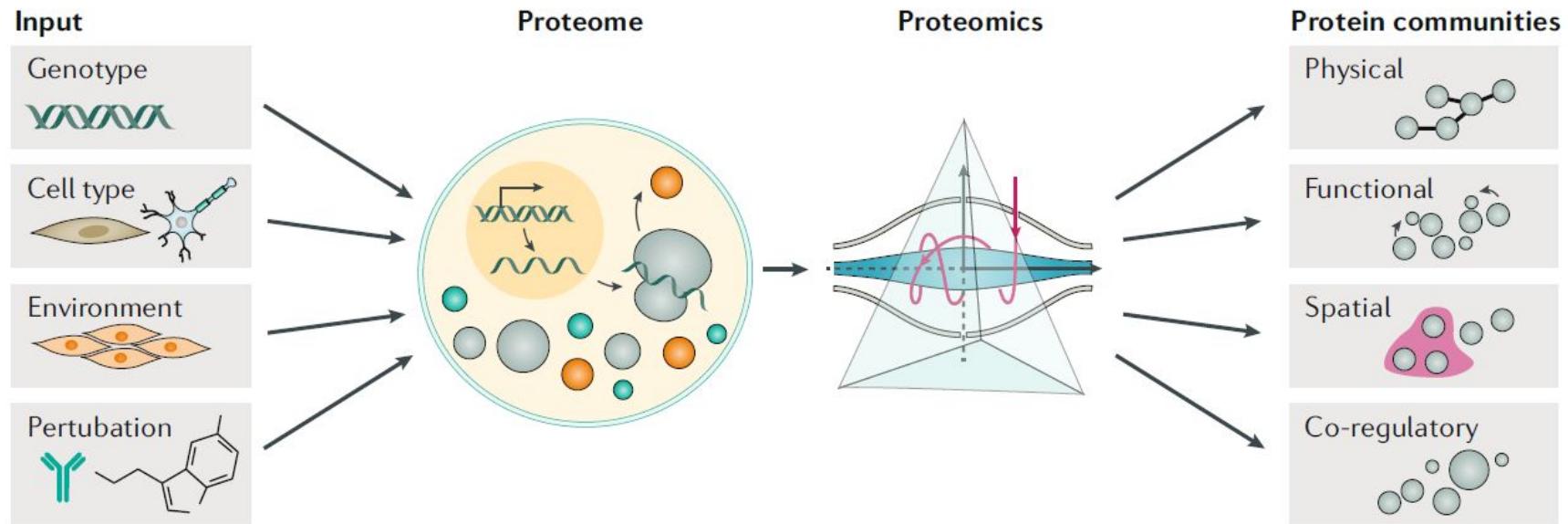
TUMOUR IMMUNOLOGY



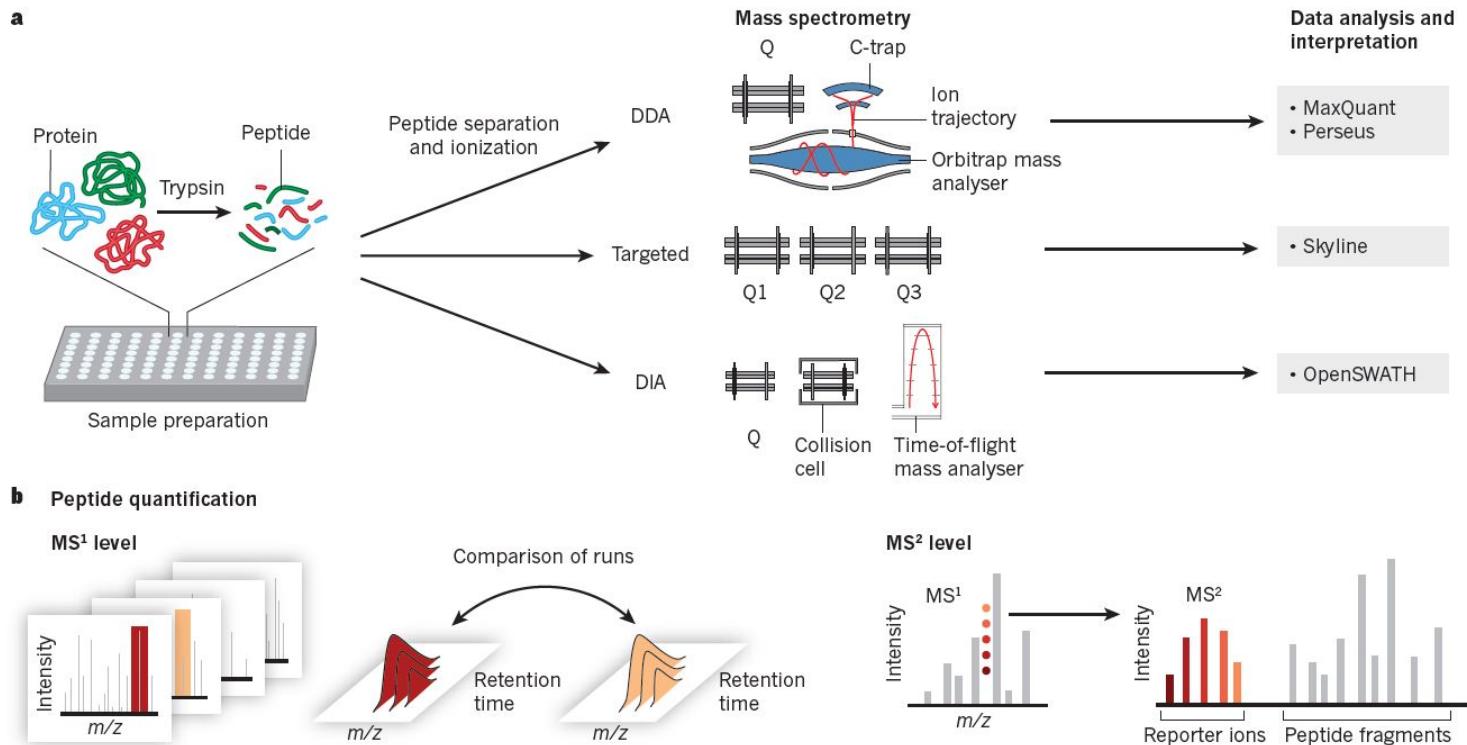
NEUROSCIENCES



Proteomics enables the elucidation of protein relations in the protein communities

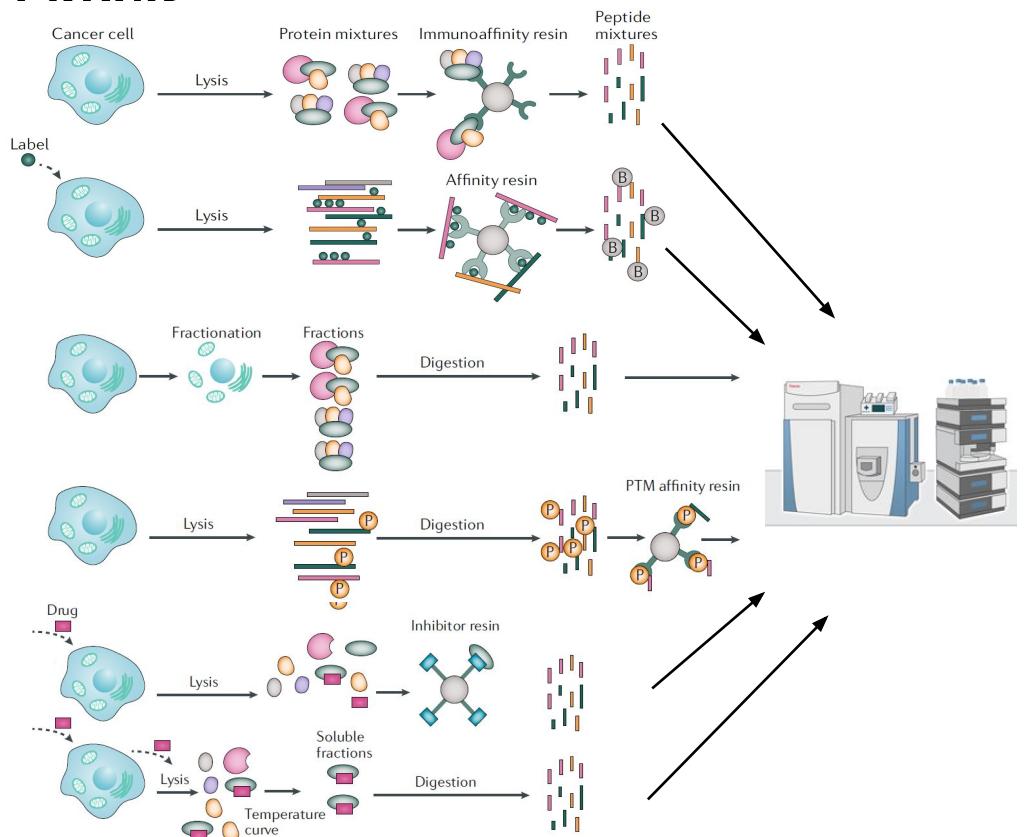


Advancement of proteomic technologies to characterize proteomes



Proteomics approaches for drug discovery

Applied for the characterization of protein communities and drug targets



Experimental approach

Affinity purification

Applications in drug discovery

Identification of drug-dependent **PPIs** and **protein-drug** interactions; characterization of **protein complex stoichiometry**

Proximity labelling

Characterization of **unbounded cellular compartments**, and **transient PPIs**

Organelle proteome profiling

Characterization of protein communities of all major **organelles** in one dataset, drug-induced **translocation**, and **secreted signaling factors**

PTM profiling

Characterization of **time- and dose-dependent signaling events** mediated by **PTM**

Chemoaffinity enrichment

Potential **drug targets** and **off-targets**

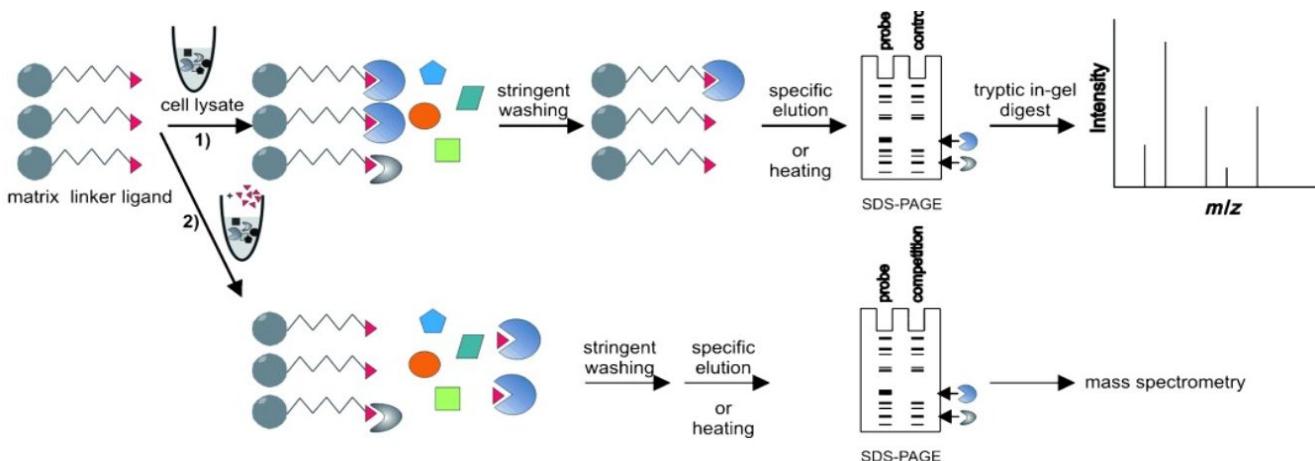
Thermal proteome profiling

Potential **drug targets** and **off-targets**

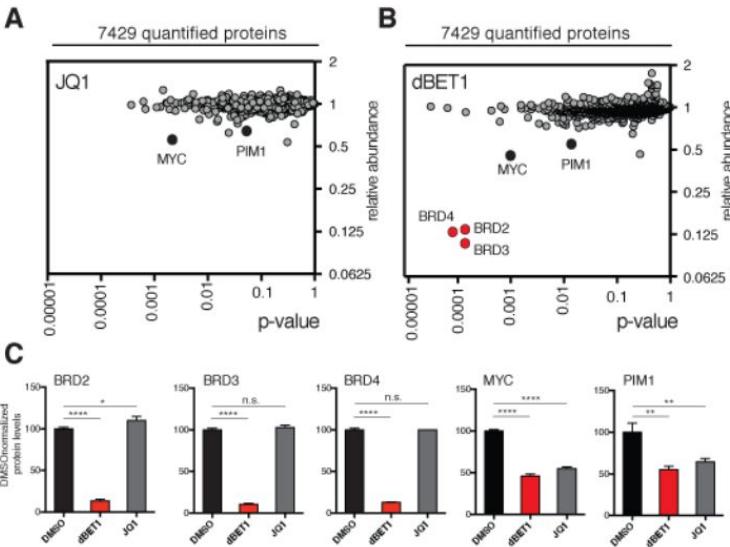
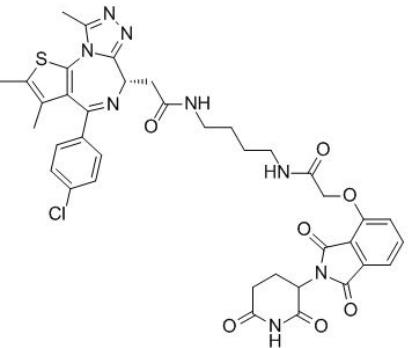
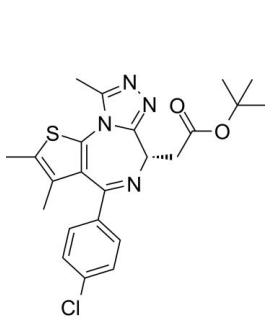
Example 1: Chemoproteomics methods for target ID



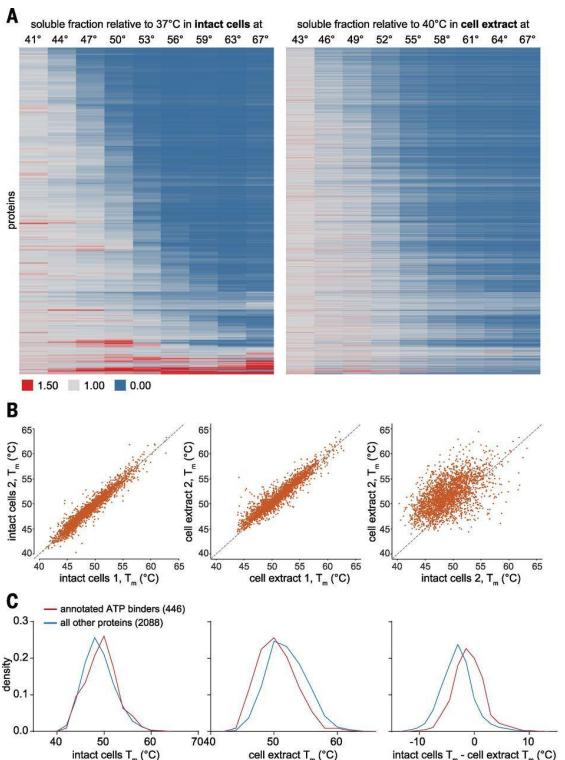
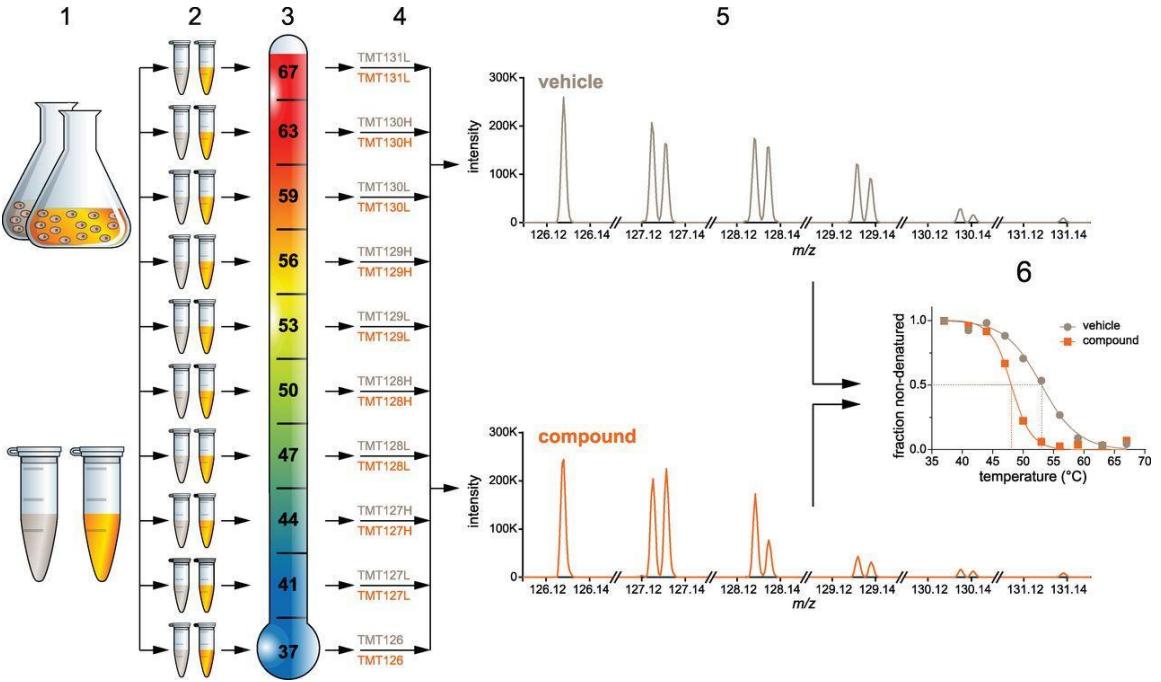
- Chemoproteomics methods are based on two principles:
(1) bait/prey and **(2) competition**.
- Commonly used methods to identify binding partners of small molecules include affinity-based profiling (shown below), activity-based profiling, SILAC, etc.



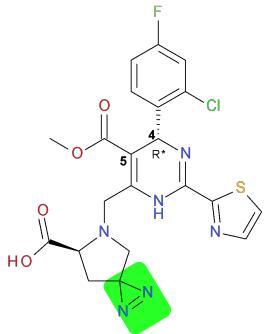
Example 2: Quantitative proteomics demonstrated selective degradation of proteins in a large set of protein mixtures



Example 3: thermal proteome profiling to track cancer drug in living cells (CETSA)

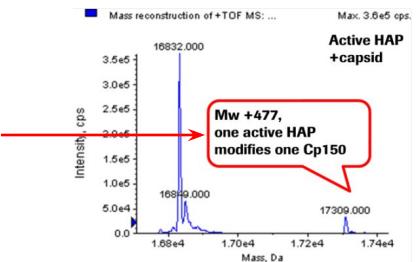


Example 4: photoaffinity labelling confirmed HBV capsid binding and mapped the small molecule binding pocket

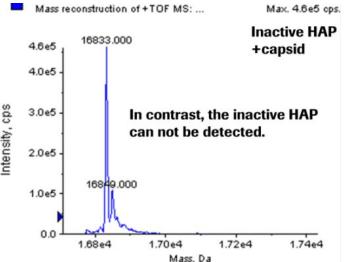


RO-A
 EC_{50} : **0.040 μM**
 IC_{50} : **0.47 μM**

+Cp150, UV, MS

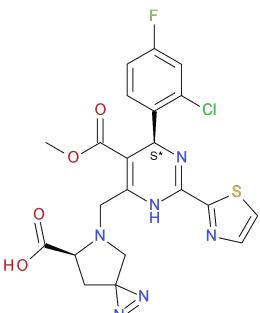
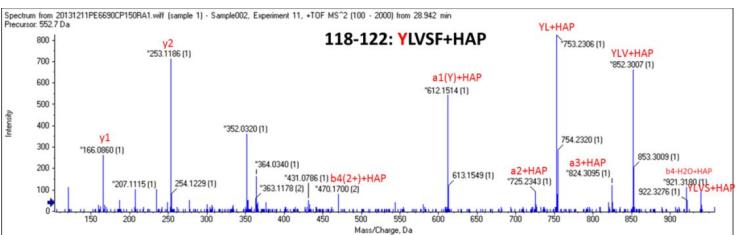


Active HAP + capsid



Inactive HAP + capsid

Proteolytic digestion/LC-MS/MS identified labelling site Y118



RO-B
 EC_{50} : **>1 μM**
 IC_{50} : **>100 μM**

More photoaffinity labelling probes identified labelling sites at R127 and Y38.

- Mass spectrometry-based proteomics allows for target and off-target identification, the understanding of PPI, and biological pathways, time-, space- and dose-dependent signaling events etc
- Future directions could be to evaluate protein communities *in vivo*, to measure stoichiometry of proteins, and to provide methods for clinical samples.

References

1. Human physiology: [Lumen Learning](#), [Exploring Nature](#), [National Geographics](#), [Platelet cells](#) (Graham Beards, CC-BY-SA 4.0), [Lymphocytes](#) (Nicolas Grandjean, CC-BY-SA 3.0), [Adipocytes](#) (Public Domain), [Hepatocytes](#) (CC-BY-NC 2.0), [Neurons and Glia](#) (Public Domain), [Blood](#) (CC 3.0), [Blood Cells](#) (By A. Rad and M. Häggström. CC-BY-SA 3.0 license)
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5. Pryor, Rosina, Povilas Norvaisas, Georgios Marinos, Lena Best, Louise B. Thingholm, Leonor M. Quintaneiro, Wouter De Haes, et al. 2019. "Host-Microbe-Drug-Nutrient Screen Identifies Bacterial Effectors of Metformin Therapy." Cell 178 (6): 1299-1312.e29. <https://doi.org/10.1016/j.cell.2019.08.003>.
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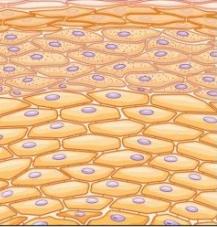
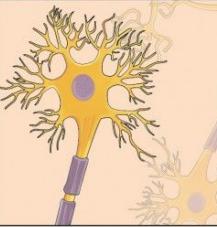
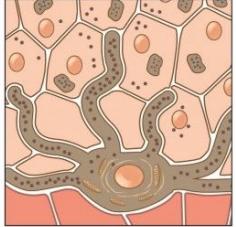
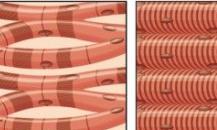
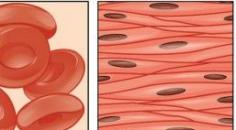
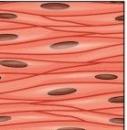
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Embryonic origins of tissues

Germ Layer	Gives rise to:				
Ectoderm	Epidermis, glands on skin, some cranial bones, pituitary and adrenal medulla, the nervous system, the mouth between cheek and gums, the anus				
	 Skin cells	 Neurons	 Pigment cell		
Mesoderm	Connective tissues proper, bone, cartilage, blood, endothelium of blood vessels, muscle, synovial membranes, serous membranes lining body cavities, kidneys, lining of gonads				
	 Cardiac muscle	 Skeletal muscle	 Tubule cell of kidney	 Red blood cells	 Smooth muscle
Endoderm	Lining of airways and digestive system except the mouth and distal part of digestive system (rectum and anal canal); glands (digestive glands, endocrine glands, adrenal cortex)				
	 Lung cell	 Thyroid cell	 Pancreatic cell		